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THE USE OF DIFFUSION CHAMBERS IN THE INDUCED

REJECTION OF A TRANSPLANTABLE RAT TUMOR

APPROVED BY

DISSERTATION COMMITTEE

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CHAPTER I

INTRODUCTION

Animals are able to recognize and react to "foreign" cells whenever such cells are placed in the internal environment of an untreated, non-related recipient. The fact that these cells or tissues are recognized as being "foreign" to the host is evidenced by their selective destruction, by activities of the host, within a relatively constant and short period of time (Medawar, 1944, 1948; Billingham et al., 1954; Billingham, 1961). Reportedly, these cells are recognized as being foreign because they are composed of materials which are different in either composition or in structure from the cells of the recipient (Campbell, 1957; Hildemann, 1958).

The stimulus for investigating the basic biologic principles involved in the transplantation of tissue from one individual to another has arisen from two areas of scientific endeavor, one being the desire to transplant normal tissue from one individual to another (Loeb, 1930), and the second, the desire to transplant neoplastic tissue in order to study neoplasia (Little and Strong, 1925). Attempts have been made since

1890 to transplant normal tissue, e.g., kidney (referred to by Calne, 1963, 1964), and skin (Levin, 1901) from one individual to another. Such attempts have led to the elucidation and a more clear understanding of the basic biological laws which govern the transplantation of tissues or organs (Russell and Monaco, 1965).

The second impetus to transplantation studies dates from 1868 (Doutrelepont, 1869) when the first recorded attempt was made to transfer a spontaneous tumor from one animal to another. Attempts such as Doutrelepont's and others (Novinsky as reported by Shimkin, 1955, 1960) soon led to the discovery of inoculable rodent neoplasms. Morau (1891, 1894) appears to have been the first to conduct a systematic survey of tumor-host relationships. He initiated the transfer of an autochthonous epithelioma from a mouse to other mice and was able to carry this tumor through seventeen serial transfers. The effects of age, sex, and predisposition to tumor growth in the first-generation offspring of tumor-bearing mice were his principle interests. He found that epitheliomas of the white mouse are inoculable into animals of the same species and that heredity plays a considerable role in the ability of these neoplasms to develop. The utility of tumor transplantation as an experimental tool thus became apparent and led to the establishment of the first rodent tumor-cell lines by Loeb (1901, 1902a, 1902b), and Jensen (1903). These investigators were able to establish cell lines of a transplantable sarcoma and a carcinoma which were carried through 150 and 19 consecutive generations, respectively (Loeb, 1901, 1902; Jensen, 1903). Attempts were made by Jensen to transplant the tumors to various species of mice but, with one exception, these were unsuccessful. Soon thereafter

additional groups attempted to transplant spontaneous neoplasms which arose in their laboratory stock animals. Ehrlich by 1906 had been able to convert 11 mouse tumors, from among a total of 94 autochthonous growths which occurred in his colony, into transplantable cell lines (Ehrlich, 1906). Bashford corroborated the findings of Jensen and Ehrlich but due to failures in attempts to transplant neoplasms in many species of animal he developed the conviction that, "The problem of the genesis of a malignant new growth must be distinguished from that of the circumstances which permit of its continued existence" (Bashford and Murray, 1904).

The investigations of Tyzzer (1907a, 1907b), and also those of Little (1914, 1920a, 1920b, 1925, 1941), developed the concept that the genetic relationship between the donor and the recipient is a matter of great importance in the transplantation of tissues. Little (1922) and later Snell (1955, 1958) developed lines of inbred mice which have been used to gain a more complete understanding of the genetic basis of transplantation. They have shown that a genetic uniformity will be developed in a closed population when the mice are mated brother x sister for approximately 20 generations (Snell, 1948). Theoretically this has occurred by progressively increasing the proportion of homozygous gene pairs present (Wright, 1933; Haldane, 1936) such that an inbred strain is said to have been attained when the proportion of such gene pairs is close to 100%. When an orthotopic skin graft is made between two individuals of such an inbred strain the graft will be permanently accepted by each individual as if it were its own tissue (Brent, 1958). Such a transplant is called an isograft.

Whenever a graft of any tissue is made between two individuals of two separate by inbred strains of the same species, the graft will be accepted for a period of time but will eventually stimulate the respective hosts to form resistance factors against the tissue of the graft. These resistance factors will then attack the graft and cause it to be destroyed or sloughed in a characteristic manner (Medawar, 1944, 1946, 1948; Billingham and Medawar, 1951). Such a graft is called a homograft and the rejection of such tissue is called a homograft rejection or reaction (Snell, 1957; Brent, 1958; Medawar, 1958). This tissue rejection has been shown to be highly specific because a second graft of tissue from the same source will be rejected in a much shorter period of time by the "sensitized" host. Such an accelerated rejection is called a "second-set" response. If similar tissues, e.g., skin, are obtained from a second, separate, unrelated source and transplanted to this same "sensitized" host they will not elicit an accelerated rejection but will be rejected as though they were primary homografts which, genetically and antigenically, they are.

The early investigators of the phenomenon of neoplasia made use of transplantation of neoplasms in order to study the neoplastic process. It has now been made clear that, instead of using transplantation to study neoplasia, they were using neoplastic tissue to study the biology of tissue transplantation. It has now been well established that neoplastic tissue obeys the same laws of transplantation as normal tissue transplants (Gorer, 1948). Autochthonous tumors which arise in one animal of an inbred strain have been shown to be freely transplantable to all other members of that same inbred strain. Such established tumors

show progressive growth which is lethal to 99-100% of the recipients (Gorer, 1938; Spencer, 1942). An additional finding was that such autochthonous tumors could not usually be transplanted from one inbred strain to another (Snell, 1958).

The inability to transplant the tumor across certain genetic lines of inheritance plus the fact that this incompatibility was linked to the inheritance of an antigen of red blood cells (Gorer, 1937, 1938; Gorer et al., 1948) and certain other inheritable morphological features of the mouse (Gorer et al., 1948) led to the definition of a genetic system which is called the Histocompatibility system (Snell, 1948; Gorer et al., 1948). Whenever an autochthonous tumor from one inbred mouse strain was transplanted to another inbred mouse strain and was subsequently rejected by the host, the strains were said to be different from each other at a hypothetical genetic locus. The first such locus discovered was called the Histocompatibility-2 locus (Snell, 1948). This genetic locus is composed of 18 alleles (Snell et al., 1964) and reportedly controls one complete portion of the antigenic make-up of tissue cells as well as an antigen of red blood cells (Gorer, 1938, 1939). The following generalization is now well established (Gorer et al., 1948; Snell et al., 1953; Snell, 1957, 1958a). Whenever the host and the tumor cells from a donor differ from each other at the H-2 locus, a tumor transplantation between the two will grow for a while and then be rejected. Tumor transplants will not grow progressively to the death of the host across an H-2 incompatibility (Snell et al., 1957). The converse of the above is also believed to be true, i.e., if a transplanted tumor grows progressively in a host, the tumor cells and the host do not differ at the H-2 locus

(Snell, 1963).

Other histocompatibility loci have been defined with the use of orthotopic skin grafts (Billingham and Silvers, 1960) or transplantable tumors (Snell, 1953) as the transplanted tissue. These have been termed histocompatibility loci H-1, H-3 (Snell, 1958b), H-4 (Snell and Stevens, 1961), H-5 and H-6 (Amos et al., 1963), and H-7, H-8, H-9, H-10 and H-11 (Snell and Bunker, 1965). Tumor transplants will grow progressively and kill the host even when an incompatibility is present in any of the loci from H-1 to H-11, with the exception of H-2 (Snell and Bunker, 1965). These, therefore, have been called the "weak" loci (Counce et al., 1956) whereas the H-2 locus is called the "strong" locus (although the H-1 and H-3 loci have been shown to be much stronger than the H-7 through H-11 loci [Snell and Bunker, 1965]). Other animals, e.g., fish (Kallman and Gordon, 1958) and guinea pigs (Bauer, 1960) have also been shown to have similar histocompatibility systems. In addition, recent reports (Bogden and Aptekman, 1962) demonstrate that highly inbred strains of rats have a histocompatibility system, analogous to that of the mouse, which controls the antigenic make-up of certain red blood cell antigens (Bogden and Aptekman, 1960, 1961) as well as that of tissue cells (Billingham et al., 1962).

The active physiological mechanisms responsible for the rejection of tissue transplants, both normal (Titus and Shorter, 1962) and neoplastic, have attracted much interest (Prehn and Main, 1953, 1954). Fundamentally, two concepts, one dealing with classical humoral antibodies and the other with cell-bound antibodies, have been proposed as an explanation of the observed incompatibilities of normal and neoplastic

transplants. Classical humoral antibodies to tumor homografts are commonly produced (Amos, 1960), but their participation in tumor rejection has been clearly demonstrated only in a limited number of tumor-host combinations (Winn, 1960). On the other hand, numerous reports implicated the macrophage (Gorer, 1956, 1958; Baker et al., 1962; Journey and Amos, 1962; Old et al., 1963); and/or the lymphocyte (Murphy, 1926; Darcy, 1952; Weaver et al., 1955; Mitchison, 1955; Mitchison and Dube, 1955; Kidd, 1950) as being the causal agents in the destruction of homografts as adjudged by their intimate contact with dying transplanted cells, either neoplastic or normal, during the course of a homograft rejection.

Several techniques have been employed in attempts to define precisely the relative contributions of humoral antibody and/or "cell-bound" resistance factors to the homograft rejection of transplanted tissue. The studies of Mitchison (1955) were particularly informative in defining the role played by "sensitized" lymphoid cells. He showed that, if recipient mice of one inbred strain are immunized by a tumor graft against the isoantigens of a different inbred donor strain, the lymph nodes in the recipient mouse draining the site of the graft can be used to "adoptively transfer" specific immunity to other mice of the same inbred strain. Placing such "sensitized" lymph nodes intraperitoneally inhibit the growth of an implant of the original tumor which is placed subcutaneously.

Other workers (Toolan, 1951, 1953, 1954a, 1954b) have utilized techniques which were designed to (a) inhibit the production of humoral antibody (Fischel et al., 1951; Kass and Findland, 1953) or (b) depress "cell-bound" resistance by the physical removal of lymphoid cells (Apolant,

1911). These workers reasoned that the persistence of a tissue graft, made under these conditions, would implicate humoral antibody and/or lymphoid cells in the normal process of tissue rejection. Cortisone has been widely used in this regard (McMaster and Franzl, 1961). It was shown that cortisone depressed humoral antibody formation (Berglund and Fagraeus, 1956; Fagraeus and Berglund, 1961), but others demonstrated that it also destroys lymphoid tissues (Dougherty and White, 1945; Bjorneboe et al., 1951; McMaster and Edwards, 1957). Therefore, a clear-cut distinction between these two possible mechanisms could not be made by the use of this hormone.

Algire (1954) has attempted to maintain the normal physiological activities of the lymphoid tissues but to also clearly delineate the influence of contact between lymphoid cells and the cells of a homograft. He suggested that filters made of a porous nitrocellulose material, when placed between the cells of a graft and those of the host, would permit the maintenance of viable tissue grafts in the body of a host but would prohibit host cells from coming into contact with and thereby destroying, if that was their nature, the grafted cells. It was further hypothesized that any soluble cytotoxic substances would be able to pass through the filter and affect the grafted cells. Using such filters and a plastic ring, he constructed a chamber, in which he placed tissue grafts, which was then, in turn, implanted in the abdominal cavity of a mouse. Since such transplants lived for long periods of time it was thereby demonstrated that the tissue cells of the graft received adequate nutrients by diffusion from the peritoneal fluids. Also, it was shown that host cells could not enter the chamber nor could tumor cells leave if the

diameter of the pores of the filter were of proper size (Algire et al., 1954; Algire and Moore, 1959). Antibody proteins, as well as nutrients, may pass freely through certain types of these filters (Holub and Riha, 1960), but in an in vitro study certain large protein molecules have been shown to be excluded by filters which have a pore size of small diameter (Gorin and Fuchs, 1961).

Utilizing Algire's diffusion chamber technique, Weaver et al. (1955) placed homografts in diffusion chambers which were then implanted in the abdominal cavity of host mice that had been specifically "sensitized" against these tissues. He was able to show that such tissues would be viable and intact when removed from chambers that had spent long periods of residence in the host. He concluded that any resistance factors present in the host were not able to cross the millipore filter and thus affect the cells in the diffusion chamber. A second critical experiment was done by placing lymphocytes of sensitized animals, along with tissues which were used to "sensitize" the donor of the lymphocytes, in a diffusion chamber which was then implanted in a non-sensitized, genetically identical host. The presence of "sensitized" lymphoid cells in the chamber was shown to have a destructive influence upon the cells of the homograft and, further, that lymphocytes were in intimate contact with the dying cells of the homograft. Algire and Weaver concluded that foreign tissues (homografts) could be maintained in an intact and, therefore, presumably viable state when implanted in diffusion chambers in a homologous host as long as cells of the host, and consequently an resistance factors strongly attached to those cells, were excluded from the chambers.

The more important question was whether tissues transplanted in such chambers functioned in a normal physiological manner. Investigations have shown that certain types of tissue were able to carry on their innate physiological functions while in such chambers. Sturgis and Castellanos (1957) have shown that ovarian tissues residing in such chambers can maintain a relatively normal estrous cycle in an ovariectomized female mouse. Other types of endocrine tissue have been transplanted in diffusion chambers with the general finding that these transplanted tissues remain intact (Stone et al., 1960; Brooks et al., 1960) and functional (Stone and Kennedy, 1962), even when they are transplanted across species lines (Stone and Kennedy, 1964). Neoplastic cells have also been shown to remain intact and viable for as long as ten months of residence in a diffusion chamber implanted in a suitable host (Amos and Wakefield, 1958).

Tests, other than the measurement of secretory function of tissue implanted in a diffusion chamber, were desired so that an estimate could be made of the viability of non-secreting tissues both normal (O'Steen, 1961) and pathological (O'Steen, 1962, 1963). Algire usually used histological tests to decide whether the tissues removed from diffusion chambers were viable. In a few instances he used the biological test of transplanting the tissue from the diffusion chamber into an animal which was genetically similar to the tissue donor (Algire et al., 1954). If the transplanted tissue grew in the host he concluded that it was viable while "in residence" in the chamber. Another test was simply the breaking of a millipore filter of an in situ chamber which contained leukemic cells. The subsequent occurrence of leukemia and the death of the host attested

not only to the viability of the cells but to their lethality as well (Algire and Moore, 1959).

The experiments described above and numerous others (Dvorak and Waksman, 1962; Parrish and Kleinfeld, 1963; Levey et al., 1963, Najarian and Feldman, 1962, 1963; Osoba and Miller, 1964; Capalbo et al., 1964) which have employed diffusion chambers very similar to those utilized by Algire indicate that the diffusion chamber itself does not have a direct effect upon tissues which are placed inside it. Specific experiments have confirmed this finding (Wilson, 1965).

However, modifying the construction of diffusion chambers by using an acrylic glue instead of a lucite-acetone mixture (as was used by Algire) has raised the question of whether tumor cells are, indeed, altered by residence in such chambers (Palmer, 1962). It has been shown that the lethality, but not the viability, of certain transplantable neoplastic tissues are greatly affected by residence in such chambers (Palmer, Keyl and Dabney, 1965). Tumor cells taken from such acrylated chambers and transplanted to susceptible Holtzman albino rats grew, causing large tumors, which then regressed. This was in distinct contrast to the demonstration that similar tumor transplants from the Algire type of chamber grew progressively to the death of the host. It was, therefore, concluded that the acrylated diffusion chamber, in some manner, altered the tumor cells so that they elicited a homograft reaction whenever they were transplanted into a highly susceptible animal.

This work strongly suggests that these neoplastic cells were "altered" experimentally so that they were recognized by the host as being "not-self" and were destroyed by an immunological mechanism. This is a provocative

idea with important potentiality. If this procedure can be extended, similar treatment of excised portions of autochthonous tumors should, upon reimplantation, elicit the formation of resistance toward the parent tumor as well as the "treated" portion. Accomplishing this, it would then seem likely that this technique could be applied to therapeutic treatment of many autochthonous tumors.

To determine whether this diffusion chamber technique of altering neoplastic cells has a broader biological application, a preliminary experiment was carried out using a different transplantable rat tumor, the Murphy-Sturm Lymphosarcoma (MSL). It was found that this neoplasm also would develop into a large tumor, which then regressed whenever it was transplanted from an acrylated diffusion chamber into highly susceptible rats of the Holtzman strain.

This present work is concerned with further investigation of the variables inherent in this type of tissue transplantation experiment, i.e., (a) those dictated by the nature of the construction of the acrylated diffusion chamber, (b) the influence of the in vivo system employed to keep the cells alive while in the chamber, (c) the nature of the resistance to the transplant which is displayed by the susceptible Holtzman rat, and (d) whether the rejection of the "altered" tumor cells substantially enhances the resistance of the animal to additional challenges of "altered" or of unaltered lethal transplants of the same neoplastic tissue.

CHAPTER II

MATERIALS AND METHODS

The presentation of this chapter will be in the following sequence: (1) materials used; (2) basic experimental plan; (3) aims and specific design of each type of experiment.

Materials

Animals

All the animals used throughout this work were from a strain of the domestic albino laboratory rat which is bred and supplied commercially from a closed breeding colony (McQuarrie et al., 1959). The nature of the breeding of this rat and the degree of homozygosity which has been obtained is of great importance to this type of investigation. The supplier (Holtzman Rat Company) has practiced selective breeding in an attempt to develop a commercial rat which would be uniform in regard to size and growth rate, gentleness, and vigor (McQuarrie et al., 1959). This has been accomplished by periodic selective brother x sister mating (i.e., inbreeding) for several generations with the subsequent establishment of a pen-bred commercial colony from the offspring (Holtzman, 1965). The selective inbreeding directed toward the above aim has concomitantly produced considerable genetic homozygosity in this population. The degree to which this has occurred has been tested (Billingham and Silvers, 1959) by the exchange of skin grafts randomly and between paired female rats with the conclusion that animals from this commercial colony are "virtually isologous" (McQuarrie et al., 1959). Other workers (Matter

et al., 1963) have also investigated, by the use of the sensitive skin grafting technique, the isologous nature of this particular strain of rat with the conclusion that autologous and homologous grafts survive for such long periods of time (both >120 days) that this strain of rat "has reached a state of histocompatibility."

Tumor

The transplantable tumor used in the experiments reported here is a lymphosarcoma which was originally developed by Murphy and Sturm (1941) from 1938 to 1941. They developed the transplantable form of the lymphosarcoma from a leukemia which they induced with 1,2,5,6-dibenzanthracene in a colony of Wistar rats. The specific tumor cell line of the Murphy-Sturm Lymphosarcoma (MSL) utilized in these experiments was obtained in 1963 from the laboratory of Hruban and Slesers of the University of Chicago. The tumor has been carried since then by re-transplantation every 9 to 18 days in the thigh muscle of female Holtzman rats (180-200 grams). The tumor homogenates used for re-transplantation, as well as those used in all of the experiments of this present work, were prepared in the following manner. Several animals which carried the tumor were sacrificed by cervical fracture. The several tumors were excised from the respective thigh muscles, debrided if necessary, and placed in a bath of sterile isotonic saline at room temperature. The tissue was minced slightly with scissors and then homogenized in a coarse-grade glass homogenizer using sterile isotonic saline as the diluent. All instruments, syringes, and glassware which were used had been thoroughly washed and rinsed. A final rinse with boiling distilled water allowed a clean technique but strict

asepsis was not attained. Since the tumors from several rats were always mixed when re-transplanting the tumor or when preparing a homogenate for an experiment, any tendency toward the creation of a subline of the tumor, different from the initial transplant, was minimized. Each new prospective host of the tumor was inoculated with one milliliter (ml) of the tumor homogenate in a thigh muscle. These animals also received an inoculation of a mixture of procaine penicillin G, benzathine penicillin G and potassium penicillin G in the opposite thigh muscle and were given additional supportive injections of the same material, if needed. The tumor inoculations developed into large tumor growths within 7 to 9 days which culminated in the death of the host in approximately 16 to 20 days. This pattern of tumor growth, i.e., its viability, lethality, and rate of growth has not changed perceptibly since the first transplantations were carried out in this laboratory, thus signifying that this tumor has been stable during the time the experiments to be described were done.

Diffusion Chambers

The diffusion chambers used in these experiments were constructed of a short cylinder 5 mm in height and 20 mm in diameter, which had a millipore filter (Type HA 0.45u diameter pore size)[1] attached to each open end, thus forming an enclosed chamber. Each cylinder used as the skeleton of the chamber had a small hole (one millimeter in diameter) drilled through one side so that a brei of cells could be introduced through a syringe needle into the chamber.

Cylinders composed of two different materials were used in certain

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[1]Millipore filter, Millipore Filter Corporation, Bedford, Massachusetts.

portions of this investigation, one being made of glass while the other was composed of an extruded plastic (lucite).

Two variations of each of these two general types of chamber were created by the use of two different adhesives when the millipore filter was attached to either the glass or the plastic cylinder. These two adhesives were either (a) one made by dissolving the extruded plastic (lucite) in acetone, or (b) a commercial adhesive which has an acrylate plastic base with ethylene dichloride as the solvent. This procedure, therefore, produced four types of diffusion chamber, i.e., (a) one composed of a glass cylinder with lucite dissolved in acetone as the adhesive, (b) one composed of a glass cylinder with the acrylate glue as the adhesive, (c) one composed of a lucite cylinder with lucite dissolved in acetone as the adhesive, and (d) one composed of a lucite cylinder with acrylate as the adhesive.

The glass or plastic cylinders were boiled in distilled water and then air dried before being used as the skeleton of a chamber. Precaution was taken to attach the millipore filters to the cylinders under clean conditions. Ethylene oxide vapor has been used extensively (Algire et al., 1954; Dvorak and Waksman, 1962) by other investigators in an attempt to sterilize the chambers after construction. But since it has been shown that ethylene oxide alters plasma proteins so that they become antigenic (Maurer, 1961) this procedure was not followed. The chambers which were constructed on any one date were stored in a covered, clean glass beaker until they were used in an experiment.

Basic Experimental Plan

The initial procedures followed in each experiment of this present work consisted of four basic steps which are presented in Figure 1 and are labeled a, b, c, and d.

A fresh homogenate, prepared as described above under the heading TUMOR, of the Murphy-Sturm Lymphosarcoma (MSL) was used in all of the experiments reported in this investigation. Immediately after the homogenate was prepared it was injected by syringe through a small hole in the wall into the chamber. This small hole was then sealed with a commercial glue[1] at which time the chamber was momentarily set aside while the Holtzman rat which was to serve as the host for the chamber was prepared. Each of these rats was anesthetized with ether, the abdominal hair was clipped and a mid-line incision was made through the skin and abdominal muscles to permit two tumor-filled chambers to be inserted into the peritoneal cavity. The incision was closed with sutures and skin clamps. This procedure was repeated until all of the chambers of any particular experiment had been implanted. The chambers were removed from these rats after approximately two to six hours at which time the tumor cells were taken from the chambers by aspiration with a syringe. The tumor homogenate from a single chamber was then injected subcutaneously in the dorsal mid-lumbar area of a single rat. This implantation of the tumor tissue allowed a method of assaying the viability and lethality characteristics of the tumor cells after short periods in the diffusion chambers. It should be emphasized that the subsequent fate of these

[1]All purpose WELDIT cement, Weldit Corporation, New York 1, New York.

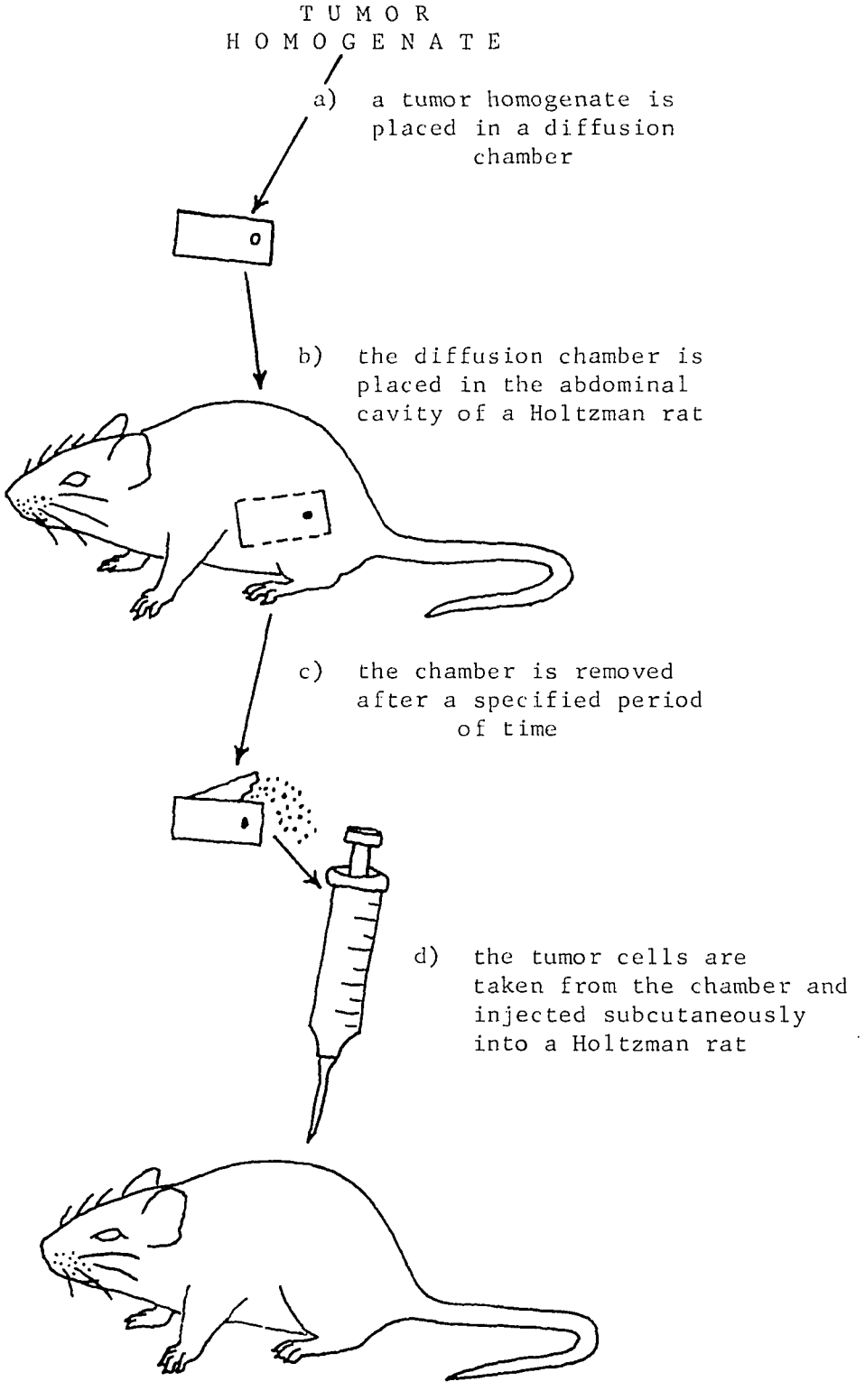

Figure 1. Basic Experimental Plan

implantations was the critical information sought in all such experiments. Each of the animals which received the material from a diffusion chamber was also given an intramuscular injection of 0.5 ml of a mixture of procaine penicillin G, benzathine penicillin G and potassium penicillin G. The rats received water and rat ration ad libitum and were checked each day. The dates of appearance of initial palpable tumor growth, initial day of apparent rejection of the tumor, or date of death of the rat due to tumor growth were recorded. Any sign of infection, although rare, was promptly treated with additional injections of penicillin.

Aliquots of each tumor homogenate prepared and utilized in the above manner were also injected into a series of untreated control rats after all of the diffusion chambers had been filled with the homogenate and implanted. The rats so injected were observed on the same schedule as the experimental rats. In this manner the viability and lethality of each tumor homogenate used in the course of these experiments were determined. The decision as to whether a rat died as a direct consequence of tumor growth was a subjective one. When animals died in the absence of apparent infection and at the same time possessed a large tumor, the death was attributed to the neoplasm.

The criterion of tumor rejection was an objective one, either the tumor killed the rat or the rat rejected the tumor. Tumors developing from implantations such as those described above grew to an appreciable size, and when rejected, followed this pattern. A black scab always formed in the skin overlying a tumor which was to regress. The scab persisted as the tumor progressively diminished in size but was finally sloughed. The wound healed with some scar formation and with little hair growth in the immediate vicinity of the scar.

Aliquots of several tumor homogenates prepared as described above were stained with trypan blue and counted in a hemacytometer to get an estimate of the number of lymphosarcoma cells per milliliter of tumor homogenate and the proportion of viable to non-viable cells (Schrek, 1936; Tennant, 1964). Representative tumor homogenates, two to four hours after preparation, had a tumor-cell count of 4 million to 6 million cells per milliliter with approximately 86% of the cells being unstained and, therefore, viable.

Aims and Specific Design of Each Type of Experiment

The specific experiments done in this work were directed toward elucidating five major questions relative to the induced rejection of the Murphy-Sturm tumor transplanted from diffusion chambers. They were:

a) Experiments designed to illustrate the influence of the acrylated chambers upon the incidence of tumor rejection (Experiments A-1, A-2, A-3, A-4).

b) Experiments to determine whether an *in vitro* system could be substituted for the animal host of the diffusion chambers (Experiments B-1, B-2).

c) Investigation of the nature of the primary resistance displayed by Holtzman rats to tumor transplants from acrylated diffusion chambers (Experiment C-1).

d) Investigation of the "resistant" state of animals which have once rejected tumor transplants from acrylated diffusion chambers (Experiments D-1, D-2, D-3).

e) Determination of the degree of natural incompatibility of the

Holtzman rat to the Murphy-Sturm Lymphosarcoma (Experiments E-1, E-2, E-3).

The details of the experiments, designed to investigate the above questions, are described below.

Influence of Components of Modified Diffusion Chambers Upon Incidence of Tumor Rejection

It was proposed to vary the several components of the diffusion chamber, when constructed, in order to determine the precise contribution of each to the rejection of transplanted tissue. Specifically it was planned to:

(1) substitute a glass cylinder for the lucite skeleton of the diffusion chamber;

(2) utilize two separate adhesives, i.e., the acrylic glue (Experiment A-1) and another composed of lucite dissolved in acetone (Experiment A-2), in construction of the glass diffusion chambers, thus giving two different types of glass diffusion chamber based upon the two different glues used;

(3) deliberately increase the amount of acrylic glue used in the construction of several different groups of lucite diffusion chamber to determine whether the rate of rejection of transplants made from such chambers would be affected (Experiment A-3);

(4) determine whether the interval of time between construction of a diffusion chamber and its use in an experiment is an important variable (Experiment A-4).

Experiment A-1 and A-2.-- Glass cylinders have been described as completely inert when used as diffusion chambers (Amos, 1961). Therefore, two different types of glass diffusion chamber were constructed, as described previously, by using the acrylic glue (Experiment A-1) or lucite dissolved in acetone (Experiment A-2) as the adhesives to attach the millipore filters to the glass rings. Chambers so constructed were utilized in several experiments which closely followed the basic experimental plan (Figure 1).

Experiment A-3 (increase of acrylic glue).-- To determine whether the incidence of rejection of tumor transplants, made from a group of acrylated diffusion chambers, was related to the amount of acrylic glue in a chamber, the amount of glue in the chamber was increased in definite steps in several groups of diffusion chamber. The first group was constructed by applying the acrylic glue only to the edge of the cylinder by which the millipore filter was attached. In the second group of chambers the amount of glue in each chamber was increased by applying the glue not only to the edge but to 25% of the surface of the inside wall of the cylinder before attaching the millipore filters. The third group had 50% of the interior wall covered in this manner while the fourth group and fifth group had 75% and 100% covered, respectively. These diffusion chambers were used in a series of experiments which were conducted as described in the Basic Experimental Plan.

Experiment A-4 (age of chamber).-- Pilot studies indicated that the age of the chamber had some influence upon the percentage of rejections of the MSL when it was transplanted from acrylated chambers. To test this aspect further, several groups of the most efficacious type of diffusion

chamber, i.e., those with 75% of the inner wall covered with acrylic glue, were constructed and set aside for different periods of time before they were used. Groups of diffusion chamber were used after they had aged for two days, or six to eight days, or 13-18 days, or 21-31 days. These chambers were employed in the basic type of experiment described previously.

Effect of an IN VITRO System of Maintaining Tumor Tissues in Diffusion Chambers Upon Incidence of Tumor Rejection

The experiments described below were designed to determine whether the same pattern of tumor growth and subsequent regression would occur whenever the tumor tissue had been incubated in an in vitro system rather than in a living rat before it was transplanted.

Experiment B-1.-- The diffusion chambers used in this experiment were constructed with the acrylic glue but did not have increased amounts of the glue on the inside surface. The acrylated chambers were filled with a homogenate of the MSL and placed in a bath of either a) Hank's balanced salt solution, or b) isotonic saline, both of which were maintained at 37°C. Neither of the in vitro solutions had plasma, serum, or amino acids added to them. Groups of tumor-filled diffusion chambers were removed from the in vitro baths after one hour, three hours, and six hours of incubation, at which time the tumor cells were taken from each chamber and injected individually into a susceptible rat.

Experiment B-2.-- In this experiment, instead of using the acrylated diffusion chamber as a container for the tumor homogenate, a series of glass test tubes were used which had been painted with the acrylic glue on their interior surface. These tubes had been prepared and set aside for different periods of time and thus were of several different ages on

the day they were used. A tumor homogenate was prepared (using Hank's solution as the diluent) and placed (one ml/tube) in the acrylated test tubes while they were maintained at 37°C. in a water bath. After certain intervals of time, viz., 30 minutes, one hour, three hours, and six hours, the tumor homogenate from each test tube of the particular age groups was removed and injected separately into susceptible albino rats. A group of glass test tubes which had not had the acrylic glue application were also used in the experiment to serve as controls.

Nature of Resistance of the Susceptible Rat to Tumor Transplants from Modified Chambers

These experiments were conducted to investigate the mechanisms responsible for the rejection, by susceptible rats, of tumors which develop from transplants of tumor cells from acrylated diffusion chambers and also to determine if possible whether this rejection is analogous to the homograft rejection of neoplastic tissue by a genetically intolerant rat. It is now established that the reticuloendothelial system in general and lymphoid tissue in particular is responsible (Russell and Monaco, 1965) for the rejection of homologous neoplastic tissues. It has also been shown that either splenectomy (Apolant, 1911) or cortisone administration (Stoerk, 1951; Stoerk et al., 1953) depresses the activity of these tissues and thus encourages grafts of neoplastic tissue. Therefore, these two techniques were used to investigate the nature of the resistance of the rat to "treated" tumor transplants.

Experiment C-1.-- A fresh tumor homogenate was prepared and promulgated according to the Basic Experimental Plan. Instead of injecting untreated animals with the tumor material from the acrylated chambers

the transplants were implanted in rats which had been treated in the following manner:

(a) one group was splenectomized one day prior to the tumor implantation and, in addition, received 20 mg/Kg. of cortisone acetate per day subsequent to the challenge with the tumor;

(b) the second group received 20 mg/Kg. of cortisone acetate per day after the injection of the "altered" tumor cells;

(c) the third group was splenectomized one day prior to the injection of the "treated" tumor homogenate;

(d) the fourth group received only the tumor transplant from acrylated chambers and were not splenectomized and thus served as controls for the tumor cells taken from such chambers.

Nature of Immunological State of Animal Which has Once Rejected Tumor Transplants from Modified Diffusion Chambers

Several techniques are available whereby the immunological state of a "sensitized" animal can be determined. One of the more sensitive and dramatic of these is provided by a second transplantation of the sensitizing tissue to the "sensitized" host. Such animals respond to such a stimulus by an accelerated rejection of the transplant, i.e., a "second-set" response (Hildemann, 1959). The fourth objective of this investigation was to determine whether those animals which have once rejected an initial transplant of "altered" tumor cells would demonstrate a "second-set" response to similarly treated tumor, i.e., now be "sensitized" to the tumor. If a "second-set" response could be elicited by this procedure,

then a second question would be investigated. It has been shown that "sensitized" animals which are challenged several times with the sensitizing antigen develop a hyperimmune state of resistance toward that antigen. It was planned to investigate the question of whether such a hyperimmune condition could be thus elicited such that the animal would resist challenges of untreated, lethal tumor cells (Experiment D-1).

An ancillary investigation was also planned. It has been been shown that animals which have once rejected a tumor transplant are strongly resistant to further challenge with that particular tumor (Snell, 1959). If the experiments described above discovered a resistant state in animals which had been challenged with "altered" tumor cells, then the experiment described below (Experiment D-2) would be attempted.

Experiment D-1.-- Albino rats, which had once rejected tumor transplants from acrylated chambers, were injected with similarly treated neoplastic tissue upon two subsequent occasions. These animals were then challenged with a lethal untreated homogenate of the Murphy-Sturm Lymphosarcoma. This experiment was carried out a second time with a different group of rats but they were inoculated with "treated" tumor material only upon one subsequent occasion after they rejected the initial implantation. They also were finally injected with a lethal tumor homogenate. The identical protocol was followed a third time but these animals were given the lethal untreated neoplastic tissue after the single initial rejection of "altered" tumor cells.

Experiment D-2.-- To determine whether the "second-set" response firmly established by the above experiments (Experiment D-1) could be easily obtunded, a group of animals which had rejected the initial challenge

of "altered" tumor implants were splenectomized and then inoculated with a lethal untreated homogenate of the MSL.

Degree of Natural Incompatibility Between Murphy-Sturm Lymphosarcoma and Holtzman Rat

For a critical assessment of the rejection of tumor transplants from acrylated chambers (by the Holtzman rat), one must compare such rejections with the inherent incompatibility of that particular tumor-host system. Specific experiments were done to determine the degree of inherent incompatibility existent between the Murphy-Sturm Lymphosarcoma and the Holtzman strain of rat (Experiment E-1). Additional data in this regard were supplied from two sources. Records were kept of the number of animals inoculated with the tumor and the number which died throughout the routine re-transplantation of the original tumor transplant (Experiment E-2). In addition, those animals which served as controls of the viability and lethality of the tumor homogenate used in each experiment of this work also provide further data along these lines. These data have been collected and labeled Experiment E-3.

CHAPTER III

RESULTS OF EXPERIMENTS

The first aim of this present work was to determine the qualities of the diffusion chamber which had an influence upon the rate of rejection of the Murphy-Sturm Lymphosarcoma transplanted from such chambers. This investigation has clearly shown that (1) an increased rate of rejection was not associated with all diffusion chambers, and (2) that use of certain types of heavily acrylated lucite chambers was associated with a greatly increased rate of rejection. Table 1 shows that glass diffusion chambers were not able to induce a change in the neoplastic tissue such that it would be rejected when transplanted, thus signifying that they were, indeed, inert. This was demonstrated by the occurrence of only two rejections out of 37 transplantations when glass chambers were used as described in Experiment A-1 and A-2. Also, it can be seen in Table 1 that there was no difference in the rate of rejection between those glass chambers constructed with the acrylic glue (5.5%) or with the adhesive made by dissolving lucite in acetone (5.2%). Investigations with lucite chambers, the results of which are described below, indicated that an increased amount of acrylic glue was required to induce the highest rate of rejection, therefore, all of the acrylated glass diffusion chambers employed in this present investigation were comparable to the lucite chambers in that they had 75% of the interior wall covered with the acrylic glue and were of the most efficacious range of age (13-18 days) for the induction of rejection. The low rate of rejection (5.5%) of transplants made from these heavily acrylated glass chambers indicates further that the acrylic glue, by itself,

did not induce an appreciable rate of rejection of transplanted tumors.

TABLE 1

GLASS DIFFUSION CHAMBERS WITH ACRYLIC GLUE (EXPERIMENT A-1) OR LUCITE IN ACETONE GLUE (EXPERIMENT A-2)

Experiment No. (Type of Glue)	Period of Residence	Number of Assay Rats	Survival Time (range in days)	Tumors rejected/ Total injected
A-1 (Acrylic)	7 hours	10	16-26[a]	1/10
A-1 (Acrylic)	16 hours	8	20-28	0/8
A-2 (Lucite in Acetone)	7 hours	10	18-26	0/10
A-2 (Lucite in Acetone)	16 hours	9	17-47	1/9

[a]Mann-Whitney U test compared with survival times of rats from Experiment C-1, Table 5, Infact Group $p = <.025$.

Preliminary work demonstrated that lucite diffusion chambers constructed with minimal amounts of acrylic glue elicited a small but increased rate of tumor rejection as compared with glass diffusion chambers. Experiments, therefore, were performed to determine the effects of increasing the exposure of the tumor homogenate to the acrylic glue. This was done by coating the interior of the lucite chamber with the acrylic glue during construction. Three different experiments were conducted which made use of lightly acrylated lucite chambers. These, as a whole, elicited a higher

rate of rejection (14.2%) than the rate of rejection (5.4%) calculated from the combined values obtained with the glass diffusion chambers (Table 1). Table 2 shows that coating the interior wall of the lucite chamber with acrylic glue greatly increased the incidence of tumor rejection.

TABLE 2

LUCITE DIFFUSION CHAMBERS CONSTRUCTED WITH ACRYLIC GLUE WITH INCREASING AMOUNTS OF GLUE ON INTERIOR WALL[a]

% of inside wall coated with acrylic glue	Type and Period of Residence	Number of Assay rats	Tumors rejected/ Total injected	p value[b]
0% (minimal acrylic glue used to attach millipore filter to lucite)	*in vivo* 2 hours	8	0/8	
	in vivo 3 hours	24	4/24	
	in vivo 6 hours	24	4/24	
25%	*in vivo* 6 hours	48	18/48	$<.02$[c]
50%	*in vivo* 6 hours	36	16/36	$<.01$[c]
75%	*in vivo* 6 hours	41	22/41	$<.001$[c]
100%	*in vivo* 6 hours	68	23/68	$<.05$[c]

[a] Experiment A-3.

[b] determined by use of Chi-square test.

[c] comparing data (i.e., Tumor rejected and Total injected) with combined data of groups with 0% glue.

The total rate of rejection from three experiments (first three lines in Table 2) was used to evaluate, statistically, the data from the experiments which made use of chambers which had increased amounts of acrylic glue used in their construction (Table 2). The rate of rejection produced when such lucite chambers (with 25% of their interior wall covered with the acrylic glue) were used was highly significant ($p = <.02$), when compared with the combined data of the three 0% groups. It can be seen from Table 2 that additional increments in the amount of acrylic glue in the chambers resulted in a greatly increased rate of rejection of neoplastic tissues transplanted from them. Those chambers which had 50% of the inside surface coated with the glue induced a rejection rate of 44% of the transplants which was highly significant ($p = <.01$). Those which had acrylic glue applied to 75% of the surface elicited a rejection rate of 53.6% which was also highly significant ($p = <.001$). The results of a Chi-square test of the data are shown in Table 2. Thus, it is apparent that an increase in the acrylic glue caused an increase in tumor rejection with the reservation that fewer rejections were obtained with chambers which had acrylic glue applied to 100% of the interior surface. Although this latter comparison is also significant ($p = <.05$), it is less so than the 25%, 50%, and 75% groups.

Preliminary experiments indicated that the age of the diffusion chamber, when used, was an important variable. This factor was investigated in a series of experiments (Experiment A-4), the results of which are presented in Table 3. These results demonstrated that there was an optimal age (13-18 days) of the heavily acrylated diffusion chambers (75% of interior surface covered with acrylic glue), during which the highest

rate of rejection was elicited. Indeed, preliminary experiments had shown that acrylated diffusion chambers, from a group which had been shown to induce an appreciable rate of rejection, lost their ability in this regard when aged two to three months. To determine the statistical significance of the age factor, a Chi-square test was made of the data from Table 3 with the demonstration that there is a highly significant ($p = <.005$) difference between the groups. The data from the 13-18 day old chambers was compared by use of the Chi-square test with the combined data of the three experiments which used lightly acrylated chambers (0% glue) as presented in Table 2. The difference between these two sets of data was highly significant ($p = <.001$).

TABLE 3

EFFECT OF AGE OF ACRYLATED LUCITE CHAMBER ON REGRESSION OF TRANSPLANTED MURPHY-STURM LYMPHOSARCOMA[a]

Age of Acrylated Chambers (in days)	Number of Assay rats	Tumors rejected/ Total injected[b]	Rate of Rejection %
2 days	8	1/8	12.5
6-8 days	41	17/41	41.4
13-18 days	80	43/80[c]	53.7
21-31 days	81	22/81	27.1

[a]Experiment A-4.

[b]Chi-square test of data of table $p = <.005$.

[c]Data from 13-18 days old group compared with total data 0% Group Table 2, $p = <.001$.

A statistical examination was also made of the length of survival of those animals which ultimately died although they received tumor transplants from the most effective type of acrylated diffusion chamber, i.e., those with 75% of the interior lucite wall coated with acrylic glue and 13-18 days of age. The Mann-Whitney U test was used to compare the survival times of this group of rats (those controls which died in Experiment C-1, Table 5) with the survival time of the rats from Experiment A-1, Group 7 hours *in vivo*. The comparison was highly significant ($p = < .025$). This demonstrates that those animals which received tumor transplants from heavily acrylated lucite chambers had a pronounced increase in their survival time over those which received transplants of the same neoplasm from glass chambers.

In summary, it can be seen that glass diffusion chambers were not only incapable of eliciting an increased rate of rejection of tumors transplanted from them but, in addition, the survival time of those rats which died was much shorter than those which received acrylated tumor tissue. The evidence also supports the concept that the acrylic glue, when used in conjunction with a lucite cylinder, provided a diffusion chamber which was able to induce the rejection of lethal tumor homogenates transplanted from such chambers. Moreover, those animals which ultimately died in experiments in which similarly treated animals rejected their transplants, had an appreciable increase in survival time.

The results of previous experiments indicated that the transplants made from acrylated diffusion chambers which were maintained for brief periods in a living rat (Table 2) would grow into large tumors which then regressed. The second question of this present work was whether *in vitro*

systems of maintaining the tumor cells while exposing them to acrylated surfaces would produce similar results. The results of the experiments exploring this question are shown in Table 4. It is readily apparent that tumor transplants taken from lightly acrylated lucite chambers (Experiment B-1) which were placed in isotonic saline or Hank's solution for brief periods (one to six hours), grew and killed the rats. The total combined rate of rejection (5.8%) of the three groups of Experiment B-1 was thereby demonstrated to be comparable to that of the glass diffusion chambers. The results from Experiment B-2 in which the tumor homogenate was placed directly in glass test tubes which had a coating of the acrylic glue revealed some slight effect of this procedure. Indeed, the group of rats challenged with the tumor homogenate removed from the 17 day old group rejected a highly significant ($p = <.012$) number of the tumors. This calculation was made by use of the Fisher Exact Probability test in comparing the rate of rejection produced by the above group and the group which received tumor transplants from lightly acrylated (Experiment B-1) chambers which were maintained in Hank's solution for six hours.

The nature of the primary resistance provoked in the highly susceptible Holtzman rat by transplants of acrylated tumor tissue was the subject of the investigations collectively titled Experiment C-1, which are presented in Table 5. Four groups of rats were inoculated with tumor material removed from heavily acrylated lucite diffusion chambers. One group was untreated and thereby served as controls of the procedures carried out on the other three groups. The other groups were: (a) splenectomized and given cortisone 20 mg/Kg/day; (b) given cortisone 20 mg/Kg/day; and (c) splenectomized one day prior to tumor implantation, respectively.

TABLE 4

EFFECT OF IN VITRO SYSTEMS OF MAINTAINING TUMOR TISSUE IN ACRYLATED CONTAINERS

Experiment No. and Type of Container	Type and Period of Residence	Number of Assay Rats	Survival Time (range in days)	Survival Time (average in days)	Tumors rejected/ Total injected
B-1 lightly acrylated lucite chamber	Isotonic saline 1 hour	8	14-23 days	17.3	0/8
	Hank's solution 3 hours	30	18-36 days	23.5	4/30
	Hank's solution 3 hours	30	17-54 days	22.1	0/30[a]
B-2 Glass test tubes with Acrylic glue					
25 days old	30 min-3 hrs	15	18-33 days	25.2	2/15
17 days old	30 min-3 hrs	20	19-48 days	26.2	6/20[a]
10 days old	30 min-3 hrs	15	12-27 days	23.0	4/15
Glass test tubes without Acrylic glue	30 min-6 hrs	14	10-28 days	21.8	2/14

[a]Fisher Exact Probability test comparison $p = < .012$.

The results of this investigation, presented in Table 5, clearly demonstrate that cortisone and/or splenectomy, alone or combined, obtunded the rejection of the tumors developed from the acrylated tumor cells. The intact controls rejected an appreciable percentage of the tumor challenges (46%) and those which died had a statistically significant ($p = <.025$) increase in their survival time. The results of these experiments strongly implicate the reticuloendothelial system as being responsible for the rejection of tumors developed from acrylated tumor tissue.

TABLE 5

RATE OF REJECTION OF ACRYLATED MURPHY-STURM LYMPHOSARCOMA BY SPLENECTOMIZED AND CORTISONIZED, SPLENECTOMIZED, CORTISONIZED, AND INTACT HOLTZMAN RATS[a]

Type of Assay Rat	Number of Assay Rats	Survival Time (range in days)	Survival Time (average in days)	Tumors rejected/ Total injected
Splenectomized and Cortisonized (20 mg/Kg/day)	10	9-25 days	18.7 days	0/10
Cortisonized (20 mg/Kg/day)	8	21-24 days	22.3 days	0/8
Splenectomized (only)	10	20-34 days	24 days	2/10
Intact	15	15-60 days[b]	32.3 days	7/15

[a] Experiment C-1

[b] Mann-Whitney U test compared with survival times of rats, Experiment A-1, Group 7 hours. $p = <.025$.

Experiment D-1 (Table 6) was carried out to determine whether those animals which had once rejected acrylated tumor tissue were thereafter resistant to similarly treated neoplastic tissue and to the lethal untreated Murphy-Sturm Lymphosarcoma as well. One group of 17 rats which rejected the initial challenge with acrylated neoplastic tissue was inoculated upon two subsequent occasions (resulting in a total of three) with similar materials. Since these two subsequent transplantations failed to grow, thereby demonstrating a "second-set" response, the conclusion was made that the recipient rats were strongly resistant to the neoplasm. This was tested by challenging these animals with the untreated lethal homogenate. Table 6 shows that this was rejected also, i.e., it did not grow at all. The homogenate was proven to be lethal by the injection of a separate untreated group of five rats which all developed tumors and died.

The above procedure was repeated with another group of 10 rats with the exception that these animals received two acrylated transplantations (one initial and one subsequent transplant) before being inoculated with a lethal tumor homogenate as can be seen in Table 6. This group also rejected the untreated tumor. This protocol was followed a third time with groups of 17 and 5 rats each (a total of 22). In this experiment the animals were challenged with a lethal untreated homogenate after they rejected the initial transplantation of acrylated tumor tissue. These animals promptly rejected the lethal tumor brei thereby demonstrating that rejection of the initial acrylated neoplastic transplant elicited a strongly resistant state in the normally highly susceptible Holtzman rat.

TABLE 6

REJECTION OF UNTREATED LETHAL HOMOGENATE OF
MURPHY-STURM LYMPHOSARCOMA BY RATS
WHICH REJECTED ACRYLATED TUMOR

Number of Rats	Times Challenged with Acrylated tumor tissue	Controls of Lethality of Tumor Homogenates used to produce Acrylated Tumor Tissue (Tumors rejected/ Total injected)	Number of rats rejecting Final Lethal untreated Murphy-Sturm Tumor	Controls of Lethality of tumor homogenate used for final challenge (Tumors rejected/ Total injected)
17	3	0/17	17	0/5
10	2	0/11	10	0/12
22	1	0/11	22	0/15

Experiment D-2 (Table 7) was conducted to determine whether the resistant condition could be easily depressed in animals which had rejected the acrylated MSL. A group of six rats which had rejected tumors developed from acrylated inoculations comprised the experimental group. Three of these animals were splenectomized while the other three were intact. These animals were then challenged with a lethal, untreated homogenate of the Murphy-Sturm Lymphosarcoma.

It can be seen in Table 7 that splenectomy does not depress the resistant condition of the rats and thereby attests to the strong resistance present in an animal which had once rejected this acrylated (treated) tumor.

In order to be able to make an accurate assessment of the rejection of acrylated Murphy-Sturm Lymphosarcoma transplants by the Holtzman rat the innate incompatibility of this tumor-host combination must be known. The experiments labeled E-1, E-2, and E-3 all provided data for such an assessment. Two specific experiments were conducted to determine the degree of susceptibility of the Holtzman rat to the untreated Murphy-Sturm Lymphosarcoma. These were carried out simply by inoculating 50 rats with an untreated homogenate of the tumor. The results of these experiments were labeled Experiment E-1 and are presented in Table 8. Those animals used as carriers of the tumor during the routine re-transplantations were recorded along with any rejections which resulted from these transplantations. These data were summed, labeled Experiment E-2 and are also presented in Table 8. Aliquots of each tumor homogenate prepared and utilized in each experiment of this present work were inoculated into untreated Holtzman rats. These rats thus served as controls for the viability and

TABLE 7

RECHALLENGE OF SPLENECTOMIZED OR INTACT RATS WHICH HAD REJECTED ACRYLATED TUMOR TISSUE WITH A LETHAL TUMOR BREI[a]

Animals Used	Individual Response to Lethal Murphy-Sturm Lymphosarcoma	Controls of Lethal Tumor Brei (Tumors rejected/ Total injected)
Three splenectomized two days prior to challenge	1) developed minimal size with rejection in 16 days	
	2) developed minimal size with rejection in 16 days	0/8
	3) did not develop tumor	
Three intact	1) developed minimal size with rejection in 16 days	
	2) did not develop tumor	0/8
	3) did not develop tumor	

[a]Experiment D-2.

lethality of each tumor homogenate. The data from these transplantations were summed and are labeled Experiment E-3 which is presented in Table 8, separately, in Table 9. Table 9, therefore, presents the sum total of the innate ability of the Holtzman rat strain to reject the tumor homogenates used in the experiments reported in this work. From Table 9 it can be seen that the Murphy-Sturm Lymphosarcoma kills such a high percentage (98.2%) of the Holtzman rat that it closely approaches the isologous condition. It can be seen in Table 8 that the degree of susceptibility of the tumor carriers agrees closely with that of the lethality controls. The conclusion can therefore be made that the Murphy-Sturm Lymphosarcoma-Holtzman rat combination is highly compatible.

TABLE 8

LETHALITY OF UNTREATED MURPHY-STURM LYMPHOSARCOMA IN HOLTZMAN RAT

Experiment	Number of Rats	Number of Rejections	Rate of Rejection
E-1 Specific Lethality Experiments #1	50	2	4.0%
#2		4	8.0%
E-2 Tumor carriers	244	4	1.6%
E-3 Lethality controls of Tumor homogenates used in each experiment	166	3	1.8%

TABLE 9

LETHALITY OF INDIVIDUAL UNTREATED TUMOR HOMOGENATES
USED IN EACH EXPERIMENT OF THIS WORK

Number of Rats	Tumor rejected/ Total injected	Survival Time (range in days)	Survival Time (average in days)
6	0/6	13-16	--
4	0/4	12-18	--
5	0/5	14-16	15.6
5	0/5	10-18	15.0
5	0/5	14-20	16.2
6	0/6	14-17	16.1
5	0/5	13-20	18.6
5	0/5	10-19	16.0
5	0/5	16-21	17.8
4	0/4	12-17	12.7
12	0/12	9-18	15.8
7	0/7	11-20	17.0
12	1/12	16-26	18.8
5	0/5	14-19	16.2
8	0/8	8-16	13.5
10	0/10	16-20	17.8
6	1/6	11-27	16.6
5	0/5	13-19	16.0
12	0/12	9-19	15.1
22	0/22	14-28	16.1
10	1/10	14-17	16.1
7	0/7	13-17	15.5
166 Total	3/166 Totals	12.5-19.4 Average range	16.08 Average

Rate of rejection 1.8%

CHAPTER IV

DISCUSSION

It is well established that the somatic cells of animals contain substances which are recognized as being "foreign" when such cells are transplanted to an unrelated recipient. Tumor homografts fail to grow, or grow temporarily and then regress, when transplanted under the above conditions. On the other hand, members of a highly inbred, i.e., isologous, strain of an animal will accept without remonstrance tumors transplanted within the strain of origin. These tumors will grow progressively and kill 99-100% of the hosts (Snell, 1953).

This present work has demonstrated that the untreated Murphy-Sturm Lymphosarcoma grows in the Holtzman albino rat and kills such a high percentage of these animals (98%) that this combination closely approaches the isologous condition, i.e., there appears to be a great degree of homozygosity in the strong histocompatibility loci (Table 9). The paramount finding of this study, however, was that transplantations of the Murphy-Sturm tumor made from heavily acrylated lucite diffusion chambers will grow, form large tumors and then regress in the untreated Holtzman rat. It was also shown that those animals which rejected acrylated tumor transplants were thereafter completely resistant to additional challenges with tumor homogenates which were lethal in untreated rats. It is possible that these results could have been produced by many separate influences or by a combination of them. Therefore, the following discussion will consider the factors which could have played a role in the causation of the rejection of these tumor transplants and will be discussed in the sequence

in which they would be operative on the tumor during a typical experiment (Figure 1).

Possible Influence of Tumor Homogenization

A brei of tumor cells in saline was used in this work because homogenization presents the same physiological condition to each cell. Thus, the supply of oxygen and nutrients to all cells falls in the same range, in contrast to a relative deprivation of oxygen and nutrients to cells in the interior of tumor blocks such as are used when transplantation is done by trocar. This constancy of cellular environment should produce a uniform growth and the results of this present work clearly show that in all transplantations, definite palpable tumors developed within one week which grew to appreciable size within two weeks. This demonstrates that the latent period between inoculation and palpability was little varied between the several types of tumor transplantations conducted in all experiments. Another advantage of an homogenate is that, whenever large numbers of animals or diffusion chambers are used, a suspension of tumor cells can be given rapidly with a higher degree of uniformity and less contamination. Early workers found it hard to reproduce their results in studies of tumor transplantation. This was due, in part, to the genetic disparity of the animals used as hosts but also to the techniques employed in transplanting the tumor (Eichwald, 1959). It seems probable that the transplantation by the use of a cellular suspension in this work accounts for much of the striking consistency in regard to both the number of tumor "takes" produced and their rate of growth.

Possible Influence of the Diffusion Chamber

The data obtained in this work almost demands the hypothesis that certain specific types of the acrylated lucite diffusion chambers have the ability, in some manner, to alter the tumor homogenate placed inside of them such that the tumor material is antigenic when transplanted to a susceptible rat (Table 2). It is readily apparent from Table 2 that all diffusion chambers constructed of a lucite cylinder and the acrylic glue did not elicit such rejections (Group 0%, Table 2). It is also very clear that glass diffusion chambers (Table 1), regardless of the type of adhesive used in their construction, are incapable of so altering the tumor homogenate. On the other hand, the use of lucite diffusion chambers which had increased amounts of acrylic glue applied to the inside wall was associated with an increased incidence of tumor rejection (Table 2). Furthermore, the ability of the heavily acrylated lucite chambers to elicit rejection of tumors was optimal during a certain age (13-18 days) of the chamber and was diminished and then lost as the chambers grew older (Table 3).

The majority of the investigations of other workers who utilized diffusion chambers for experimental use make no mention of the effect of the chamber upon tissues placed in them. Indeed, specific experiments conducted by Wilson (1965) "gave no evidence" that "commercial plexiglass diffusion chambers have any effect upon neoplastic (lymphoma) cells placed in them," although he was aware that such chambers may not be "completely inert biologically." Such plastics as the methacrylates (e.g., plexiglass, lucite) are known to be toxic to animals (Amos, 1961) and to be carcinogenic (Merwin and Algire, 1959; Merwin and Redman, 1963; Shelton et al., 1963). It is not surprising then, that these plastics might affect neoplastic cells (or

others) which are placed in containers constructed of them.

Additional suggestive evidence as to the activity or ability of methacrylate to induce antigenic changes in isologous neoplastic cells is supplied by the data of several investigators. Essentially, their technique consisted of pre-treating inbred mice with heavily irradiated tumor cells native to the particular inbred strain. When groups of these mice were subsequently challenged with increasing doses of living tumor cells, similar to the irradiated cells, a slight level of resistance to such viable isologous inoculations was observed. The authors interpreted this slight resistance as being of "doubtful significance" and thought that "it can be most plausibly attributed to a non-specific effect similar to that found in the previous work on methylcholanthrene-induced sarcomas" (Klein et al., 1963). The important factor in the present context is that they used an "....irradiation chamber of methacrylate polymer...." (Revesz, 1955) as a container for the tumor cells as they were being irradiated. Exposure to the methacrylate in this manner may have, to a minimal degree, changed the tumor cells in a manner similar to that proposed in this present work.

The mechanism by which certain of the acrylated chambers used in this work might have affected the contained neoplastic cells is unknown. However, two aspects of the phenomenon are clear; (a) a combination of the lucite chamber with increased amounts of acrylic glue is necessary to elicit the highest rate of tumor rejection, and (b) the period of time which the tumor homogenate must be in the chamber is relatively short (2 to 6 hours). Inherent in these two observations is the possibility that components (e.g., proteins or lipoproteins) of the surface of the neoplastic

cells, which are not originally antigenic may be quickly changed by contact with, or exposure to, the acrylated lucite chamber such that they will become antigenic when introduced into a susceptible animal. It may be that some substance found in the wall of the acrylated lucite chamber has formed a complex with certain portions of the neoplastic cell.

The possibilities in this regard may have been increased by the use of a tissue homogenate with the resulting "complex mixture of substances" (Davies, 1962). Davies (1962) has demonstrated that the antigens which dictate H-2 specificities can be extracted from such "complex mixtures" and this gives credence to the speculation, now proposed, that the use of tumor homogenates in this present work may have brought a component of the MSL cell into contact with the acrylated chamber wall so that a combination between the two occurred to give an antigenic complex for transplantation.

There are numerous substances supplied in the neoplastic "complex mixture" described by Davies which might play the role in forming the antigen. The most prominent possibilities would be; (a) a substance which behaves as an H-2 antigen when transplanted to a homologous host, (b) other antigens which are dictated by weaker autosomal loci, e.g., H-1 or H-3, (c) some other cellular substance which would not normally be antigenic and is not governed by histocompatibility loci. In this present work, the tumor rejection response was strong and, especially so since it was induced in a system that behaved, normally, as if it were isologous. Therefore, the involvement of an histocompatibility antigen similar to the H-2 in the mouse must be suspected. A comparison of the length of survival of those animals which received tumor transplants from heavily acrylated lucite chambers with the length of survival of animals which received tumor transplants

from glass diffusion chambers revealed a difference between the two groups which was highly significant ($p = <.025$). A lengthened survival time of those rats which received tumor transplants from acrylated chambers, but were unable to reject the tumor and died, could be due to a weak (H-1 or H-3) antigenic response of the host to the tumor. The third possibility listed above would be the formation of a complex between particles supplied by the chamber and substances of the tumor cell which would not naturally be antigenic when transplanted to a homologous host and are not controlled by histocompatibility genetic loci. A mechanism such as this would be analogous to the antigenicity of skin homografts. Those grafts which differ from the host at the H-2 locus are strongly antigenic and are sloughed rapidly while those which differ only at the "weak" H-1 or H-3 loci are weakly antigenic and are sloughed only after a prolonged period of time.

It has been shown that the surface of neoplastic cells is very different from that of normal cells (Lowick et al., 1961) and that the adhesiveness of neoplastic cells is much less than normal cells (Abercrombie and Ambrose, 1958, 1962). It has also been shown that surface phagocytosis plays a major role in defense mechanisms of the body which are directed against "foreign" antigens (Smith and Wood, 1958). Indeed, it has been said that "Phagocytosis undoubtedly contributes to the rejection of homografts of tumor cells ...." (Bennett et al., 1964). Therefore, another speculative possibility is that some component (e.g., a polymer) of the acrylated chamber attached itself to an intact tumor cell resulting in an increase in the immune attractiveness of the cell; i.e., the nature of the surface of the neoplastic cell may have been changed such that phagocytes

could adhere to the cell and phagocytize it. Evidence from several sources supports such a speculation. It has been shown that methyl cellulose (a polymer), when pre-injected in large amounts (900 mg) into Sprague-Dawley albino rats, will increase the rate of rejection of the Murphy-Sturm Lymphosarcoma from the innate level of 23% to a level of 95%. These workers (Lazar and Lazar, 1962) attributed this increase to a non-specific stimulation of the reticuloendothelial system (RES) which they believed came about by the uptake of the methyl cellulose by reticuloendothelial cells. These authors did not consider two pertinent points. It would have been necessary for the tumor cell, or some portion of it, to also have been taken up by the RES for the stimulation of those mechanisms which are responsible for developing a resistance to "foreign" antigens. Furthermore, they did not consider the possibility that the methyl cellulose polymer may have combined with the Murphy-Sturm tumor cell *in vivo* and, by doing so, favored its phagocytosis. Indeed, it has been shown that such polymer formation can occur *in vivo* (Haddow, 1959) and many reports demonstrate that intracellular polymer formation can occur (Hendry *et al.*, 1951). This hypothesis is further supported by the demonstration that haptens, such as picryl chloride, which have highly reactive chemical groups, can combine *in vivo* with the body proteins producing complexes which can then act as antigens (Chase, 1955).

It has been shown that antibody formation can take place as long as the antigen persists in those cells which are able to produce antibody (Haurowitz, 1965) but cannot continue in the absence of antigen. Such persistence of "foreign" antigens in immunologically competent cells is supposedly due to the lack, by those cells, of enzymes which can degrade

the antigenic complex (Campbell, 1957; Crampton et al., 1963). In this regard it would appear highly likely that any complex formed between an exogenous, plastic polymer such as used in this work and constituents of a tumor cell, if engulfed by an immunologically competent cell, would resist degradation and act as an antigenic complex. Indeed, it has been shown that there are many synthetic materials which, although never encountered in nature, are able to elicit production of specific antibodies when combined with naturally occurring non-antigenic substances.

One other possibility must be considered. All components of cells which could serve as antigens are ultimately determined by the deoxyribonucleic acids (DNA) of that cell. Thus the change which occurred in the tumor cells, rendering them antigenic to the untreated rat might have been some change in the DNA. However, none of the results of the present work offers any direct information on this speculation.

Possible Influence of the In Vivo and In Vitro Method of Maintaining the Neoplastic Tissue

Results from the experiments in which the tumor-filled chambers were placed in an in vitro tissue culture system (Table 4) indicate that (a) the tumor cells can be maintained in such a system, and (b) minimal rejection of these tumors occurred when they were implanted into a rat.

These results could be construed to mean that the living albino rat is necessary as the host of the diffusion chamber in order to obtain an appreciable rate of tumor rejection. However, the possibility of using an in vitro system to maintain the neoplastic cells viable (while exposing them to an acrylated lucite chamber) has not been explored to a sufficient degree especially since the in vitro systems used in this present work

were not fortified with serum, plasma, or amino acids. An exogenous source of protein, or other material which is supplied by an in vivo system, may be necessary to the cells to enable them to be recognized as "foreign" when the cells are transplanted to an isologous host.

Since the animals used as an in vivo host for the tumor-filled chambers are virtually incapable of responding to untreated tumor during routine transplantation of the tumor, it would appear that they would not possess any specific immune mechanisms capable of affecting the tumor cells in a diffusion chamber in the time available. It is also evident that the rat hosts do not affect, in a non-specific manner, such implantations. This was demonstrated by the transplantation of tumor cells from glass diffusion chambers or from certain types of lightly acrylated lucite chambers (Table 2). Such transplants developed into tumors at the same rate as those taken from heavily acrylated chambers. Transplants from both types of acrylated chambers developed into palpable tumors within a similar range of time, i.e., the latent periods were the same. The tumors from both types of chamber continued to develop at the same rate and a disparity in the tumors only became evident when an appreciable percentage of those from heavily acrylated chambers decreased their growth rate and began to regress.

The experiments in which an acrylated glass test tube was used as the container of the tumor homogenate while exposing it to an acrylated surface are difficult to interpret due to the small sample size. However, certain types of acrylated test tube (17 days old group) gave an increased incidence of tumor rejection and a lengthened survival time of those animals which died (Table 4). When the rate of rejection associated with the

17 day old acrylated test tube group is compared with that from the lightly acrylated diffusion chambers which were maintained in Hank's solution for six hours, the difference between the two was highly significant ($p = .012$). This indicates that it should be possible to maintain tumor tissues in an *in vitro* system and still be able to elicit the growth and rejection of transplants from acrylated containers. A further indication along this line is that the age of the acrylated test tubes which gave the highest incidence of tumor rejection was 17 days which was near the age of the group of acrylated diffusion chambers (13-18 days) associated with the highest rates of tumor rejection.

Possible Influence of the Assay Rat

The Holtzman albino rat which was used as a biological assay of the viability and lethality of all transplantations of neoplastic tissue made in this present work has been shown to be highly susceptible to the untreated Murphy-Sturm tumor (Table 8) or to MSL taken from lightly acrylated diffusion chambers (Table 2) or glass diffusion chambers (Table 1). The experimentally induced variations from the normal pattern of tumor growth or lethality must, perforce, reflect a significant change in the tumor since the rat has been demonstrated to be isologous and was not pretreated. An additional finding enlarges this conclusion. The number of tumor cells inoculated into each rat in the experiments reported here was high (approximately 4 to 6 million). Large numbers of neoplastic cells have been shown to overcome any minor antigenic differences elicited or innate in neoplastic tissues (Stoerk *et al.*, 1953; Snell, 1959; Klein *et al.*, 1963). Experiments conducted by Stoerk (Stoerk *et al.*, 1953) are germane to this point. These workers were able to obtund the incidence

of "takes" of challenging doses of 250,000 cells of the Murphy-Sturm Lymphosarcoma by prior injections of homologous lymphoid tissue (e.g., spleen, thymus). This decrease in the number of "takes" was eliminated by large numbers of tumor cells or by injecting the tumor cells simultaneously with the homologous tissues. Therefore, any rejection of fully developed tumors which resulted from inoculations of large numbers of tumor cells by untreated rats in this present work indicates that a major antigenic difference was present.

Work from several additional sources lends credence to the demonstration in this present work that the Holtzman rat (an animal which cannot normally remonstrate against the MSL) was stimulated antigenically by inoculation with neoplastic tissue from heavily acrylated chambers. It has been shown that the pre-injection of lyophilized tumor material into an animal will immunize against subsequent challenges with the lethal homograft (Kaliss, 1952). Dabney (1960) has conducted experiments similar to Kaliss' which are particularly germane to this present work due to the strain of rat (Holtzman) and the tumor (Murphy-Sturm Lymphosarcoma) employed. He pre-injected Holtzman rats with lyophilized Murphy-Sturm Lymphosarcoma which developed into tumors in a small number of animals which subsequently regressed. These animals were then injected with a lethal homogenate of the Murphy-Sturm tumor which grew and killed all of the rats so treated, thus demonstrating that the Holtzman rat could not be immunized by this procedure against subsequent challenges with the untreated MSL. Therefore, the demonstration in this present work that an inoculation of MSL cells from heavily acrylated chambers developed into large tumors, which then regressed, strongly indicates that such an

inoculation was highly antigenic.

The nature of the primary resistance developed in the albino rat to tumor transplants from acrylated lucite chambers was explored in a series of experiments (Table 5). The demonstration that splenectomy and cortisone administration, when combined in experimental rats, obtunded the rejection of acrylated-tumor transplants strongly implicates the reticuloendothelial system as being responsible for the rejection by intact rats of similarly acrylated (control) transplantations. Cortisone administration alone was almost as effective in obviating this rejection response as was splenectomy and cortisone combined. Splenectomy alone was the least effective method of depressing the rejection of acrylated tumor tissue. These results are interpreted to mean that the spleen, which is believed to be primarily involved with the production of soluble humoral antibodies (Silverstein, 1964), was not involved to a high degree in the rejection of tumors developed from "treated" tumor cells.

This present investigation has shown that the Holtzman rat which has been induced to reject an initial challenge of the "treated" Murphy-Sturm tumor was resistant to any additional challenges with a lethal homogenate of this tumor (Table 6). Moreover, it was found that several sensitizing inoculations of the "treated" tumor cells were not necessary for the production of this immune condition but that those animals which reject the initial acrylated transplant are thereafter resistant to the untreated lethal homogenate. In addition, it was shown that ablation of the spleen of an animal which had rejected an initial transplantation of the MSL prior to challenge with a lethal tumor brei did not obtund the animal's ability to reject the tumor. A certain few animals permitted this lethal

tumor brei to grow for a short while but then quickly rejected it. This can be compared with the demonstration that splenectomy alone could obtund the rejection of a significant percentage of the initial inoculations of acrylated tumor tissue (Table 5).

Such findings as the above lead directly to the conclusion that the lethal MSL tumor has been attenuated such that it acted as a "foreign" antigen when transplanted to a highly susceptible Holtzman rat. Many attempts have been made to attenuate tumor tissue, e.g., by the use of Freund's adjuvant (Hartveit, 1962a, 1962b) such that it would be antigenic and thereby stimulate the host to reject in situ autologous neoplastic tissues. A search of the literature reveals that such attempts have been singularly unsuccessful. It would appear that the present experiments are highly unique in that not only was the tumor attenuated such that it was rejected, but also that it provoked the susceptible host to develop such a strongly resistant state that untreated, proven lethal, Murphy-Sturm tumor was prohibited from growing -- thereby attesting to the highly immune condition of this once susceptible rat.

CHAPTER V

SUMMARY AND CONCLUSIONS

Experiments which were conducted in this work have produced the following findings and conclusions.

(1) This investigation clearly showed the Murphy-Sturm tumor to be lethal in such a high percentage of the Holtzman rat recipients that it approaches the isologous level of compatibility. It is therefore concluded that the Holtzman rat-Murphy-Sturm Lymphosarcoma combination is such a highly compatible system that the rat has little, if any, innate ability to resist this tumor.

(2) Preliminary work had suggested that this tumor could often be made antigenic by residence in diffusion chambers constructed with lucite and an acrylic glue. Therefore, a series of experiments were carried out which used lightly acrylated lucite chambers as containers for the tumor homogenate. The use of such chambers produced a slight but definite increase in the rate of rejection when transplants were made from them. These results implicated the combination of acrylic glue with lucite in the increased rate of rejection of transplants.

(3) To explore the possible relationship between the amount of acrylic glue in a lucite chamber and incidence of rejection of tumors transplanted from such chambers, a series of experiments was done in which lucite chambers were used which had increased amounts of the acrylic glue on their interior wall (25%, 50%,

75%, and 100% of surface covered). The results of these experiments were highly significant ($p = <.05$ to $<.001$) and clearly indicated that the use of the heavily acrylated lucite diffusion chamber was associated with a dramatically increased rate of rejection. The conclusion, therefore, is made that the heavily acrylated lucite chambers altered the neoplastic homogenate placed in them such that it would be recognized as being "foreign" when transplanted into the normally susceptible rat. This recognition allowed the development of resistance against the tumor such that the tumor was attacked and rejected.

(4) Experiments were performed to determine the influence of glass diffusion chambers, if any, upon the incidence of rejection of transplants of the Murphy-Sturm Lymphosarcoma (MSL) made from such chambers to Holtzman rats. Transplantations of the MSL made in this manner grew into tumors which killed 95% of the Holtzman rats. Therefore, the conclusion was made that the glass diffusion chambers were inert and had little ability to affect neoplastic tissues placed in them.

(5) Other glass diffusion chambers were used which had 75% of their inside surface coated with the acrylic glue. It was postulated that the behavior of transplantations made from a diffusion chamber such as this would reflect the influence of the acrylic glue. Transplants made from these chambers also grew and killed a high per cent of the Holtzman rat. These results allow the conclusion that the acrylic glue as used in a glass diffusion chamber did not encourage an increase in the rate of

rejection of MSL transplants.

(6) Preliminary experiments suggested that the age of the diffusion chamber was a critical variable in the elicitation of a high rate of rejection of tissues exposed to the chambers. This factor was investigated with the finding that a certain optimal time (13-18 days) after chamber construction was the most efficacious in eliciting rejection while chambers of 21-31 days of age had begun to lose their capacity to elicit rejection and chambers of great age (two to three months) failed to elicit rejection of transplants made from them. The conclusion is drawn that the ability of the acrylated chamber to provoke rejections of transplanted tumor cells is labile, appearing rather quickly and diminishing with time.

(7) The nature of the primary resistance toward the acrylated tumor tissue evoked in the Holtzman rat was investigated by splenectomy, cortisone administration, and by a combination of these procedures. The results of these experiments showed that combining splenectomy with cortisone administration abolished the development of resistance to tumor from acrylated chambers. Large doses of cortisone were also highly effective while splenectomy alone was least effective in diminishing the development of resistance to the tumor. These results support the conclusion that the reticuloendothelial system of the rat is responsible for the development of resistance to acrylated tumor tissue.

(8) An attempt was made to determine whether rats which once rejected the acrylated tumor tissue were resistant to subsequent

challenges with similarly treated tumor and to untreated homogenates which were proven to be lethal by transplanting aliquots to untreated Holtzman rats. It was discovered that rats which rejected acrylated tumor tissue were thereafter resistant to all subsequent challenges of the treated or the untreated Murphy-Sturm lymphosarcoma. It is therefore concluded that inoculations of the acrylated tumor with subsequent rejection of the tumor developed from such transplants evokes a strongly resistant or immune condition in once susceptible Holtzman rats.

(9) In several experiments in which plain and acrylated glass test tubes were used instead of diffusion chambers, the results were inconclusive. A minimal rate of tumor rejection suggests that this procedure should be tested further.

(10) It was found that the survival time of rats, which received tumor transplants from heavily acrylated chambers, was much increased even though the animal eventually died due to the tumor. The survival time of these animals was compared with the survival time of those rats which died when injected with a tumor transplant from glass diffusion chambers. A highly significant difference was found between the two groups in that those which had received the acrylated material lived for longer periods of time. This result was obtained even though the latent period between tumor inoculation and palpable tumor growth was not prolonged in this group over other groups. The conclusion is made that those animals which received the acrylated tumor, and which ultimately died, exerted a minimal resistance to the tumor. It is possible

that this resistance may be a response of the host to antigens, analogous to the H-1 or H-3 antigens of the mouse, which were experimentally altered so that they are antigenic.

The results from this present work support the hypothesis that the acrylated diffusion chambers attenuated the Murphy-Sturm Lymphosarcoma placed in them such that the tumor was antigenic when transplanted to a host rat which normally does not react against the untreated tumor. The suggestion is made that this technique might have general application to many neoplastic tissues and might also be applicable to autochthonous tumors.

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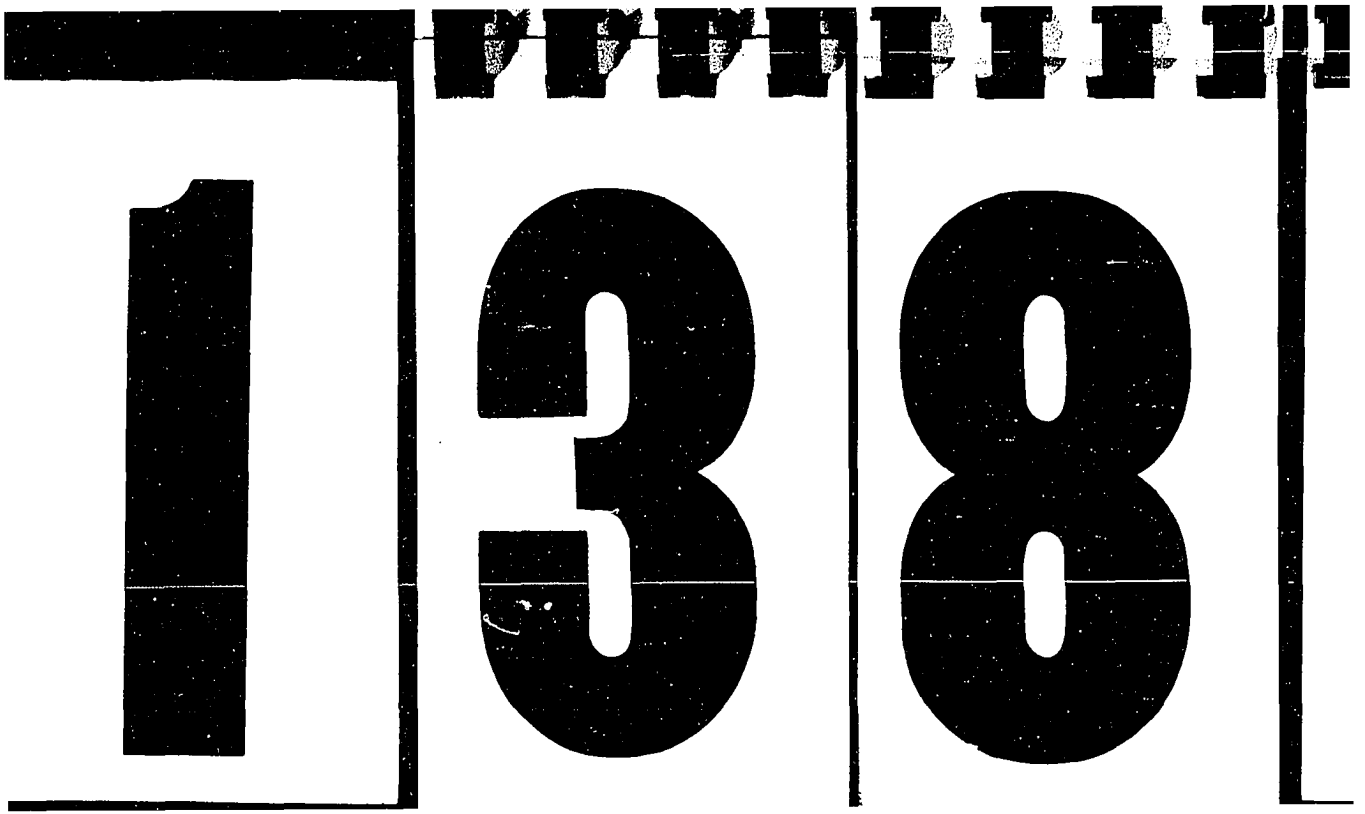
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